PHYTOCHEMICAL SCREENING AND EVALUATION OF THE DIURETIC ACTIVITY OF SEED EXTRACT OF *QUERCUS LEUCOTRICHOPHORA*

SYNOPSIS OF THE THESIS

Submitted by

Chinu Kumari

Registration No 18RCPPHP003

Under supervision of

Dr. Amit Chaudhary

In Partial Fulfillment of the Requirement for the Degree of

DOCTOR OF PHILOSOPHY

SCHOOL OF PHARMACY
FACULTY OF PHARMACY
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CHAIL CHOWK, MANDI (H.P.) – 175028

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DECLARATION

I hereby declare that the dissertation synopsis entitled **"Phytochemical Screening and Evaluation of the Diuretic Activity of Seed Extract of *Quercus leucotrichophora*"** to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

Place: chail-chowk.

Date: 10/04/2021

Signature of the Scholar

Chinu Kumari

Forwarded by: Concern Supervisor

Place: chail-chowk.

Date: 10.04.2021

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12/4/2021

Introduction:

Plants are used to maintain the health of human beings and also prevent, diagnose, treat, and heal physical and mental illnesses. These plants are also known as Medicinal plants or Medicinal herbs. Medicinal plants can be defined as the plants that possess therapeutic properties or exert a beneficial pharmacological effect on the human or animal body. Plant medicine is also posses the diuretic property and traditionally used in the treatment of many diseases.[1]

The word diuretic has a *Greek* stem, *Diu* (through) *ovpein* (to urinate), and a diuretic is defined as any substance that increases urine flow and thereby water excretion.[2]

Diuretic compounds that stimulate the excretion of water are potentially useful in most of the disorders including those exhibiting oedema such as congestive heart failure, nephritis, toxemia of pregnancy, premenstrual tension, and hypertension.[3] The diuretic plants stimulate the lymphatic circulation and improve the detoxification. A diuretic decreases the cardiac workload, oxygen demand, and plasma volume and also decreases blood pressure. Thus diuretics play an important role in hypertensive patients.[4]

Definition: Diuretics are the drug that increases the rate of urine formation together with natriuresis. Diuretics are used to adjust the volume and composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis.[5]

Mechanism of Diuretics:

Loop diuretics, inhibit the sodium-potassium-chloride cotransporter in the thick ascending limb. This transporter normally reabsorbs about 25% of the sodium load. Therefore, inhibition of this pump can lead to a significant increase in the distal tubular concentration of sodium, reduced hypertonicity of the surrounding interstitium, and less water reabsorption in the collecting duct. This altered handling of sodium and water leads to both diuresis (increased water loss) and natriuresis (increased sodium loss).

Thiazide diuretics, which are the most commonly used diuretic, inhibit the sodium-chloride transporter in the distal tubule. Because this transporter normally only reabsorbs about 5% of filtered sodium. These diuretics are less efficacious than loop diuretics in producing diuresis and natriuresis. Their mechanism depends on renal prostaglandin production.

Potassium-sparing diuretics. Unlike loop and thiazide diuretics, some of these drugs do not act directly on sodium transport. Some drugs in this class antagonize the actions of aldosterone

(**aldosterone receptor antagonists**) at the distal segment of the distal tubule. This causes more sodium (and water) to pass into the collecting duct and be excreted in the urine. They are called K^+-sparing diuretics because they do not produce hypokalemia like the loop and thiazide diuretics.

Carbonic anhydrase inhibitors inhibit the transport of bicarbonate out of the proximal convoluted tubule into the interstitium, which leads to less sodium reabsorption at this site and therefore greater sodium, bicarbonate and water loss in the urine. These are the weakest of the diuretics and seldom used in cardiovascular disease. Their main use is in the treatment of glaucoma.[6]

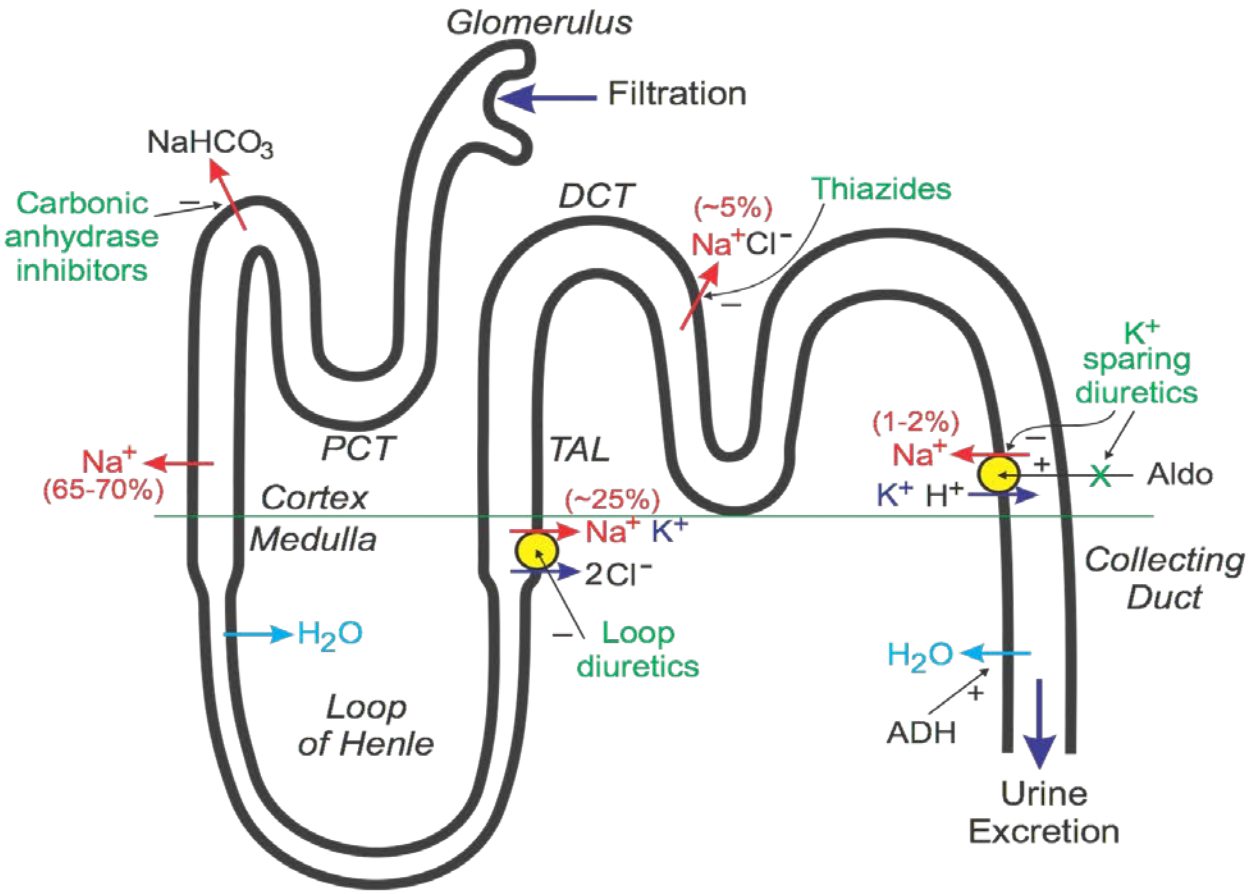


Figure 1: Mechanism action of diuretic [30]

Classification of Diuretics:[5][6]

Table 1. Classification,use and adverse effect of diuretics

Sr. No	Class of diuretics	Example	Uses	Adverse Effects
1.	Thiazide and related diuretics	Bendroflumethiazide Chlorthalidone Cyclopenthiazide Indapamide Metolazone	Edema, Hypertension, Diabetes insipidus, Hypercalciuria.	Allergic manifestations, Hyperuricaemia, Hyperglycaemia, Hyperlipidemia, Hypocalcaemia, Magnesium depletion.
2.	Carbonic anhydrase inhibitors	Acetazolamide	Glaucoma, To alkalinize urine Epilepsy, Acute mountain sickness, Periodic paralysis.	Acidosis, Hypokalaemia, Drowsiness, Fatigue, Abdominal discomfort, Hypersensitivity reactions-fever, rashes.
3.	High Ceiling/Loop Diuretics	Furosemide Bumetanide Torasemide	Edema, Acute pulmonary edema, Cerebral edema, Hypertension, Hypercalcaemia of malignancy	Hypokalaemia, Hypomagnesemia, Dilutional hyponatraemia, Hyperglycemia, Hypovolemia,Hypercholesterolemia.
4.	Osmotic diuretics	Mannitol	Barbiturate poisoning, Threatened acute renal failure, Cerebral edema, Raised intraocular pressure.	headache, nausea, chills, polydipsia, confusion and pain in chest, Exessive amount of mannitol can cause cellular dehydration; pulmonary edema in patient with CHF and hyponatremia.
5.	Potassium-	Amiloride	To treat oedematous	Hyperkalaemia,

	sparing diuretics and aldosterone antagonists	Triamterine Spironolactone Eplerenone	conditions including liver cirrhosis, Combined with thiazides, they can be used to treat refractory oedema, In combination with thiazides or loop diuretics they are used to treat hypertension.	Triamterene causes – Rise in blood urea, nausea, dizziness and muscle cramps.

Natural Diuretics: The synthetic diuretics exhibit various adverse effects such as electrolyte imbalance and metabolic alterations etc[2] due to these side effects we prefer natural diuretics.

Plant profile:

Common names: Himalayan oak, blackjack oak, white oak Banjh oak, Banj oak (Uttarakhand), and Ban oak (Himachal).

Biological source: The drug obtained from the leaves, seeds, and bark of plant ***Quercus leucotrichophora***

Family: Fagaceae

Description:

Quercus species represents an important group of evergreen or deciduous trees from temperate and tropical climatic areas. *Quercus leucotrichophora* is a medium-sized tree, usually attaining about 15 m in height, occasionally 24 m. Young seedlings up to two years old are very shade-tolerant, and thereafter the tree needs moderate to full light. It needs moderately fertile soils, growing well on clays derived from shale's and clay loams, but does not do well on dry sites. The seed ripens between November and March, according to the locality, but the ripe seed may remain on the trees for several months. In the Himalayas, the banj oak is found at altitudes between 1500 and 2400 m above sea level.[7]

Morphology:

Quercus leucotrichophora A. Camus has a full, rounded canopy. The bark is a smooth tan-brown at first, becoming lightly furrowed and corky with age. The leaves are alternate, elongated ovals with jagged teeth. Young leaves are pink-purple in color and the upper surface turns deep green as it matures, while the lower side is silvery grey due to the presence of white hairs (hence the

name leucotrichophora, meaning "which has white hairs"). The male inflorescences (catkins) are borne on the tips of the branches, while the tiny round female flowers are borne at the base of the leaves. The fruits are orange-tan, marble-sized acorns.[8]

Medicinal uses:

The dry resin of *Quercus leucotrichophora*, Baanj (local name) is taken with water for stomach pain.[9] The corm of the plant is used as an astringent and diuretic.[10] The seeds of the plant are used in the treatment of a urinary disorder.[11]The bark of the plant (25g bark in 100ml) is used in the form of gargle in tonsillitis.[12]

In Jammu, Kashmir, and Ladakh (India) revels that the bark of *Quercus leucotrichophora* is used to cure toothache and piles, leaves are used as an astringent and in the treatment of diarrhea while the gum resin is used as Stomachache. [13]

In Uttarakhand uses gum resin of the plant by making its paste to cure Gonorrheal, asthma, hemorrhages, diarrhea, and dysentery.[14]

Quercus leucotrichophora is used in the treatment of gonorrheal and digestive disorders in Garhwal Himalaya.

The fruit powder is used for the treatment of urinary infection in the region of North Pakistan

Quercus leucotrichophora seeds are used in the treatment of snakebite. The dried gum powder of the plant used with milk during weakness in the body.[8]

Review of literature

The Diuretics are the drug that increases the rate of urine formation together with natriuresis. Diuretic is used to adjust the volume and composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephritic syndrome, and cirrhosis.

The Banj oak (*Quercus leucotrichophora*) belonging to the family Fagaceae is an evergreen tree of approximately 40 m height and commonly found throughout the Himalayan region with a latitudinal range from 800 to 2300 m. The plants of the genus *Quercus* contain various types of plant secondary metabolites including terpenoids, flavonoids, phenols, steroids, tannins (ellagitannins, and hydrolyzable tannins), and many others. [15]

Semwal P *et al*., (2018) researched on Screening of phytoconstituents and antibacterial activity of leaves and bark of *Quercus leucotrichophora* A. Camus from Uttarakhand Himalaya and repoted that both the extracts (leaves and bark) of *Quercus leucotrichophora* have antibacterial activity against gram positive (Staphylococcus aureus, Bacillus Bacillus subtilis and Streptococcus pyogenes) and gram negative (Escherichia coli, Pseudomonas aeruginosa) bacterial strains. [16]

Chauhan P.S. *et al*., (2017) reported that the *Quercus leucotrichophora* has multipotential medicinal plant and it is used to treat various ranges of diseases in the traditional system of medicine. Different parts of the plant such as a corm, seeds, leaves, fruit, and dry gum resin are used to cure various ailments such as urinary infection, toothache, piles, diarrhea, asthma, hemorrhages, dysentery, astringent, diuretic, tonsillitis, and snakebite. [14,16]

Joshi A.K. *et al*., (2017) reviewed that the *Quercus leucotrichophora* is also a valuable plant especially for the treatment of GIT disorders and urinary tract infections. The seeds act **as** astringent and diuretic agents and are also used in the treatment of indigestion, diarrhea, and asthma in humans. [8]

Sati A. et al., (2016) study on "Chemical composition and antimicrobial activity of fatty acid methyl ester of Quercus leucotrichophora fruits" and said that the extract of the fruits of Q. leucotrichophora contains the higher amount of saturated fatty acids than unsaturated fatty acids. They confirmed that the fruits of Quercus leucotrichophora contain a higher percentage of the fatty acids and has potential as an antibacterial principle for clinical application. Thus, based on this, they can conclude that the fruits of Quercus leucotrichophora have high-quality potential for use in the soap, cosmetics, and pharmaceutical industries. [17]

Mishra S. et al., (2013) said that Diuretics like Mannitol, Thiazides, Furosemide, and Ethacrinic acid are used as synthetic drugs nowadays. But these diuretics had adverse and side effects. These synthetic diuretics typically inhibit potassium retention. [18]

Wile, D. et al., (2012) reviewed on Diuretics and reported that the diuretics are used in conditions where there is oedema and those which are non-oedematous. Oedema occurs in heart failure, renal failure, the nephrotic syndrome and with ascites in hepatic cirrhosis. The non-oedematous states include hypertension, nephrolithiasis, hypercalcaemia and diabetes insipidus. Oedema occurs in heart failure, renal failure, the nephrotic syndrome and with ascites in hepatic cirrhosis. The non-oedematous states include hypertension, nephrolithiasis, hypercalcaemia and diabetes insipidus. [19]

Raghavendra HL., (2009) calculated that herbal therapy is one of the best practices to overcome the illness. The herbal medicines are in great demand in the developed as well as developing countries for primary health care because of their wide range of biological activities, higher safety margins, and lesser costs. Public, academic, and government interest in herbal medicines is growing exponentially due to the increased incidence of adverse drug reactions and the economic burden of the modern system of medicine. These herbal based medicines are formulated from cultivated as well as wild plants. [20]

Butler J et al., (2004) studed on "A relationship between heart failure treatment and development of worsening renal function among hospitalized patients" and said that the Diuretic compounds that stimulate the excretion of water are potentially useful in many disorders including most of those exhibiting oedema such as congestive heart failure, nephritis, toxemia of pregnancy, premenstrual tension, hypertension and also play an important role in hypertensive patients and pulmonary congestion.[21]

Nadkarni KM. et al., (2000) reported that the crud drug being always available easily in abundance, comparatively cheaper with negligible side effects, and has frequently been described to patients of all age groups. The multiple therapeutic actions and uses of these drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books and Pharmacopoeias.[22]

Rationality: .

As per the literature review, it was found that current diuretic drugs are potent but had a lot of adverse and side effects. So, a new drug is required with maximum potency and a negligible side and adverse effect. As my study comprises of natural origin drug which may have less side effect with maximum potency. It was also found from various kinds of literature that the Quercus leucotrichophora has diuretic property. However no such experimental work was found on diuretic activity of Quercus leucotrichophora. So, experimental work is required for ensuring the diuretic activity of Quercus leucotrichophora.

Aim and objective:

Aim: Phytochemical screening and evaluation of the Diuretic activity of seed extract of *Quercus leucotrichophora.*

Objective:

- Phytochemical screening of *Quercus leucotrichophora.*
- Characterization of compound of *Quercus leucotrichophora.*
- Pharmacological evaluation of Diuretic activity of extracted compound of *Quercus leucotrichophora*
- Statistical analysis of different Pharmacological parameters

Proposed Research Methodology

1. **Literature survey:** The literature collects from the review, papers, books, and Google Scholar, PUBMED, MEDLINE, NCBI, Science Direct.
2. **Selection of plant:** As per the literature review, it was found that current diuretic drugs are potent but had a lot of adverse and side effects. So, a new drug is required with maximum potency and a negligible side and adverse effect. As my study comprises of natural origin drug which may have less side effect with maximum potency. From various kinds of literature, it was found that till now less experimental work had done on the diuretic property of ***Quercus leucotrichophora.*** Hence I selected this plant.
3. **Collection of plant material:**

The plant will be collected from the region of District Mandi, Himachal Pradesh, India.

4. **Chemicals and Instruments:**

Table. 2 List of chemicals:

Sr.no	Chemicals
1.	Ethanol
2.	Methanol
3.	Acetone
4.	Ethylacetate
5.	Dichloromethane
6.	Potassium mercuric iodide
7.	Potassium bismuth iodide
8.	Tannic acid
9.	Ferric chloride
10.	Vanillin hydrochloride
11.	Chloroform
12.	Sodium hydroxide
13.	Lead acetate
14.	Sulphuric acid
15.	Ninhydrin

16.	Fehling A and Fehling B

Table. 3 List of Apparatus, Instruments, and Glassware:

Sr.no	**Apparatus / Instruments/ Glassware**
1.	Soxhlet
2.	Percolator
3.	Weighing machine
4.	Metabolic cage
5.	Beaker
6.	Test tube
7.	Measuring cylinder
8.	Glass rod

The *Quercus leucotrichophora* is use as test solution,test substance, plant extract and test drug in this synopsis

5. Extraction of plant:

Extraction in chemistry is a separation process used to separate a substance from a matrix**.** In recent years active principles from plants have been isolated or obtained as purified products of precisely known potency and stability. The plant contains chemical substances some of which provide relief from a variety of diseased conditions. The solvent used for extraction is known as "Menstruum" and the undissolved residue left behind after the process is called "Marc".

The process of drug extraction is.

Penetration of the solvent into the drug

↓

Dissolution of the constituent

↓

Outward diffusion of the solutions from the cells

↓

Separation of dissolved portion

As per literature the extraction of Medicinal plants is done with 1. Cold aqueous percolation 2. Hot aqueous extraction (decoction) 3. Solvent extraction (cold / hot)

5.1 Decoction: In this process, the crude drug will be boiled in a specified volume of water (1;4) for a defined time. The volume will be reduced to 1/4th the original, then it will be cooled and strained / filtered. This procedure will be suitable for extracting watersoluble, heat-stable constituents.

5.2 Percolation: In this process, the solid ingredients are moistened with an appropriate amount of the specified menstruum. The mixture will be allowed to macerate in the closed percolator for 24 h.

5.3 Soxhlet apparatus: The Soxhlet apparatus based on the process of "Hot Continuous Extraction".

In this method, the finely ground crude drug will be placed in a porous bag or "thimble" made of strong filter paper, which will be placed in the chamber of the Soxhlet apparatus. The extracting solvent in the flask will be heated, and its vapors condense in the condenser. The condense extractant drip into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in the chamber rises to the top of the siphon tube, the liquid contents of the chamber siphon into the flask. This process will be continuous and will carry out until a drop of solvent from the siphon tube will not leave a residue when evaporated.

The advantage of this method is that large amounts of the drug can be extracted with a much smaller quantity of solvent. This affects a tremendous economy in terms of time, energy, and consequently financial inputs. On a small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on a medium or large scale.[16,23,24]

6. Phytochemical investigation of different extracts: The plant extracts used as a test solution [25] [26]

Table. 4 : Methods of phytochemical investigation

Sr.no	Test	Procedure	Observation
1.	**Test for Alkaloids**	**a) Mayer's Test-** Test solution 1ml+ few drops of Mayer's reagent (Potassium mercuric iodide solution)	The color change precipitate indicates the presence of Alkaloids

		b) Dragendroff's Test- 1ml test solution + few drops of Dragendroff's reagent (Potassium bismuth iodide solution)	The color change precipitate indicates the presence of Alkaloids
		c) Wagner's test- 1mL of extract + 1mL of potassium iodide (Wagner's reagent) will be added and shaken.	Appearance of reddish brown precipitate signifies the existence of alkaloids
		d) Hager's test- 1mL of solution of an extract + 1mL of saturated ferric solution (Hager's reagent) will be added and shaken.	Formation of yellow-colored precipitate indicates the existence of alkaloids
		e) Tannic acid Test- Test solution 1 ml + few drops of 10 % tannic acid solution	The color change indicates the presence of Alkaloids
2.	**Test for tannins**	**a) Ferric chloride (FeCl3) Test-** About 0.5 mg of dried powder of plant extract + 20 ml water in a test tube, boils, and filters. A few drops of 0.1 % ferric chloride solution will be added	The brownish-green or blue-black coloration will indicate the presence of Tannins
		b) Gelatin Test- About 1ml test solution + 1 % gelatin + 10 % sodium chloride solution	The white precipitate will indicate the presence of Tannins
		c) Vanillin hydrochloride Test- Test solution+ few drops of vanillin hydrochloride reagent	The purplish- red color will indicate the presence of Tannins
3.	**Test for Steroids**	**a) Liebermann Buchard test-** Test solution 1 ml + few drops of acetic anhydride, boil and cool + add concentrate sulphuric acid	The brown ring at the junction of the two layers and the green layer in the upper layer will indicate the

			presence of Steroids
4.	**Test for Flavonoids**	**a) Alkaline reagent test-** 1 ml test solution + few drops of sodium hydroxide solution	The yellow coloration which will disappears on the addition of dilute HCl indicates the presence of Flavonoids
		b) Lead acetate Test- Test solution 1 ml in a test tube + add few drops of lead acetate solution	The yellow color precipitate will indicate the presence of Flavonoids
5.	**Test for Terpenoids**	**a) Salkowski Test-** Test solution 1 ml + 2 ml chloroform + few drops of sulphuric acid. Shake well and allow to stand for some time	The reddish-brown color will indicate the presence of Terpenoids.
6.	**Test for Proteins**	**a) Ninhydrin Test-** Test solutions boil with 0.2 % solution of ninhydrin	The violet color will indicate the presence of Proteins
		c) Xanthoproteic Test- 1mL of the extract will be taken and placed it into a test tube. Then few drops of nitric acid will be added and shaken.	Emergence of yellow-color indicates presence of protein
7.	**Test for Reducing sugars**	**a) Fehling Test-** Test sample 1 ml + 0.5 ml of Fehling A and Fehling B solutions and boil	The brick-red coloration indicates the presence of Reducing sugars
8.	**Test for Saponins**	**a) Froth test-** Test solution 1 ml will place in a test tube containing water and shake well	Note for a stable froth that persists for at least 2 min.
9.	**Test for Phenols**	**(a) Ferric chloride test-** 1mL solution of an extract will be taken and placed into a test tube. Then 1% gelatin solution	Formation of bluish-black color indicates the presence of phenols.

		containing sodium chloride will be added and shaken.	
		(b) Lead acetate test- 1mL solution of an extract will be taken and placed into a test tube. Then 1mL of alcoholic solution will add, followed by dilution with 20% sulfuric acid. Finally, solution of sodium hydroxide will be added.	Formation of red-to-blue color signifies the occurrence of phenols.
		(c) Gelatin test- A solution of plant extract will be placed into test tube followed by 2mL of 1% gelatin solution and shaken.	Appearance of white precipitate indicates the presence of phenols.
		(d) Mayer's reagent test (potassium mercuric iodide test) - To a solution of plant extract, 1mL of Mayer's reagent will be added in an acidic solution.	Manifestation of white precipitate shows the existence of phenolic compounds.
10.	**Test for glycosides**	**(a) Bontrager's test (modified).** One gram of the crude extract will be first weighed, placed into a test tube, and dissolved in 5mL of dilute hydrochloric acid. Then 5mL of ferric chloride (5%) solution will be added. The mixture will be shaken and placed over water bath. Then the mixture will allow boiling for 10min, cooled, and filtered. Afterward, the mixture will be then extracted again with benzene. Finally, equal volume of ammonia solution will be added to benzene layer.	Appearance of pink color indicates the presence of anthraquinone glycosides.

		(b) Legals test- 1mL of an extract will be taken, and then an equal volume of sodium nitroprusside will be added followed by few quantity of sodium hydroxide solution and shaken.	Formation of pink-to-blood-red precipitate signifies the existence of cardiac glycoside.
		(c) Keller–Killiani test- 2mL of the extract will be taken and diluted with equal volume of water. Then 0.5mL of lead acetate will be added, shaken, and filtered. Again, the mixture will be extracted with equal volume of chloroform, evaporated, and dissolved the residue in glacial acetic acid. Then few drops of ferric chloride will be added. Again, the whole mixture will place into a test tube containing 2mL of sulfuric acid.	Emergence of reddish brown layer that turns bluish green implies the presence of digitoxose.

7. Fractionation

Fractionation is a process of separation of plant extracts into various fractions. It further segregates the fractions into portions comprising a number of compounds. The process continues until pure compound is isolated.[26] When several solvents are required for the fractionation, they should be added according to the order of increasing polarity. Fractionation techniques are basically classified into physical or chemical method.

Chemical methods

This extraction method is based on the type of functional groups possessed by a compound in the given mixture. Separation or purification can be achieved by chemical reactions using appropriate reagents. [26]

Physical methods

Physical methods used in separation of compounds from mixtures include separation funnel method, chromatographic techniques, fractional distillation, fractional crystallization, fractional liberation, and sublimation. [26,27,28]

Separation funnel method: When four different solvents (*n*-hexane, chloroform, acetone, and *n*-butanol) are selected, fractionation begins by moistening or complete dissolution of crude extract with 250mL of water. This is followed by transfer into a separating funnel, shaken, and allowed to settle. Furthermore, to 250mL of *n*-hexane, the least polar solvent was added and shaken. The content can settle, and the bottom of the separating funnel opened to remove the aqueous layer. The remaining content in the separating funnel was poured into a clean container to get *n*-hexane fraction. Equal volume of *n*-hexane was added again, shaken, and separated. The addition continued until after adding *n*-hexane and shaken no reasonable quantity of extract appeared to move into the *n*-hexane portion. Similar cycle was performed for chloroform, acetone, *n*-butanol to get chloroform, acetone, and *n*-butanol fractions. The remaining portion left after the fractionation is termed as residual aqueous fraction (RAF) as the crude extract was first dissolved in water. [26,27,28]

Fractional distillation: This is a process of separating or purifying compounds from a mixture. It is usually used in separation of hydrocarbons such as crude oil, citral, and eucalyptol. Purification is achieved based on the differences in their boiling points. Fractional distillation apparatus is constructed in such a manner that when heat is applied each compound will evaporate and separates at its boiling point. Consequently, each compound fractionated will condense and collected as a separate entity through several siphons attached to fractional distillation apparatus.

Fractional crystallization: Large numbers of compounds that exist naturally in plant extracts are crystal in nature. Separation is achieved via formation of crystals during concentration of an extract using heat or refrigeration.

Fractional liberation: This method is suitable for separating compounds that can easily form precipitate from the mixture. The precipitate is usually formed by changing the compounds into their salt form. Fractional liberation is commonly applicable in purification cinnamon alkaloids.

Sublimation: This method involves changing from solid to gaseous state without passing through liquid state. Substances such as camphor and volatile oils when heated get separated and converted directly into gas. [26,27,28]

7. Characterisation of components:

7.1 Thin-layer chromatography: Thin-layer chromatography is a "solid-liquid adsorption" chromatography. In this method, the stationary phase is a solid adsorbent substance coated on glass plates. The silica gel will be utilized as adsorbent material. In this method, the mobile phase travels upward through the stationary phase The solvent travels up the thin plate soaked with the solvent employing capillary action. During this procedure, it also drives the mixture priorly dropped on the lower parts of the plate with a pipette upwards with different flow rates. Thus the separation of analytes is achieved. This upward traveling rate depends on the polarity of the material, solid phase, and of the solvent . In cases where molecules of the sample are colorless, fluorescence, radioactivity, or a specific chemical substance can be used to produce a visible colored reactive product so as to identify their positions on the chromatogram. The formation of a visible color can be observed under room light or UV light. The position of each molecule in the mixture can be measured by calculating the ratio between the distances traveled by the molecule and the solvent. This measurement value is called relative mobility and expressed with a symbol Rf. Rf. value is used for the qualitative description of the molecules. [29][30]

7.2 High-performance Thin Layer Chromatography (HPTLC):

High Performance Thin Layer Chromatography is capable of separating organic compounds. Principle of HPTLC have similar approach and employ the same physical principles of TLC (adsorption chromatography) i.e. the principle of separation is adsorption. The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster. Thus, the components are separated on a chromatographic plate.

HPTLC is a powerful tool for rapidly comparing several samples in parallel, checking for adulteration, monitoring purity and stability, and quantifying marker compounds. Because of its many benefits the technique is used in various application fields. The analyte includes herbal

drug, food, forensic, biotechnology, cosmetics, pharmaceutical, chemical, environmental samples[29][30]

8. Study the Diuretic activity of plant:

8.1 Animals: Experimental protocols and procedures will be approved by the Institutional Animals Ethics. The care and handling of the animals are in accordance to the CPCSEA guidelines for laboratory animal use and care, Government of India.

8.2 Animal Husbandry Conditions

The animals will be housed under controlled temperature (24 ± 2∘ C) and relative humidity (45% ± 10%).

Table 5: Animal Husbandry Conditions

Temperature	24 ± 2∘ C
Relative humidity	45% ± 10%
Air changes	10 to 15 air changes per hour.
Housing	Standard polypropylene rat cages with stainless steel top grill were used to house the animals. The cages were washed, clean paddy husk was used as the bedding material.
Sanitation	Bedding material will be changed on alternative days.
Light/dark cycle	12-hourly
Feed & water	Standard pelleted feed and Filtered water was provided ad libitum. The diet and water were routinely analyzed for any contaminants that could reasonably by expected to affect the purpose or integrity of the study

8.3 Preparation of the test substance : The test substance will be calculated on the basis of mg/kg .

8.4 Evaluation of diuretic activity

8.4.1 Lipschitz model:

Thirty rats will be deprived of water but not food for 18 h. Their urinary bladders will be emptied by gentle compression of the pelvic area and by pull of their tails. Each of these rats will be orally administered with 15 ml of isotonic saline (NaCl, 0.9% w/v) to impose a uniform water

load. Forty-five minutes later, these rats will randomly assigned into five groups (N = 6 per group) and treated orally in the following manner. The Test dose1, Test dose 2, Test dose 3 are *Quercus* extract at different concentration.

Table. 6 Treatment groups for diuretic activity

Group I	Negative control (distilled water)
Group II	Test dose 1
Group III	Test dose 2
Group IV	Test dose 3
Group V	Standard drug (Furosemide)

Each of these rats was individually placed in metabolic cages and cumulative urine output will be determined at hourly intervals for 5 h. The colour of urine will also note. In an attempt to ascertain the broad mechanisms of action, the urine collected from group 1 (control) and group 4 will be subjected to the following investigations: pH by pH meter, Na+ and K+ levels by flame photometry , osmolarity by Osmometer, glucose and proteins (Reagent strips). [31]

8.4.2 DOCA-salt induced hypertension in rats.

The diuretics are use in treatmet of hypertension. The hypertensive effect is increased by salt loading and unilateral nephrectomy in rats.

Procedure: The rats weight 250–300 g will be anesthetized with ether. Through a flank incision the left kidney is removed. The rats will be injected twice weekly with 20 mg/kg s.c. desoxycorticosterone acetate in olive oil for 4 weeks. Drinking water will be replaced with a 1% NaCl solution. Blood pressure starts to rise after one week and reaches systolic values between 160 and 180 mm Hg after 4 weeks. [32] [33]

Table.7 Treatment groups for hypertensive activity

Group I	Negative control (Normal saline solution)
Group II	Disease control
Group II	Test dose 1
Group III	Test dose 2

Group IV	Test dose 3
Group V	Standard drug (Furosemide)

8.5 Parameter assessments

During study protocol different parameters will be assessed as Physical assessments, hemodynamic assessments, biochemical assessments,

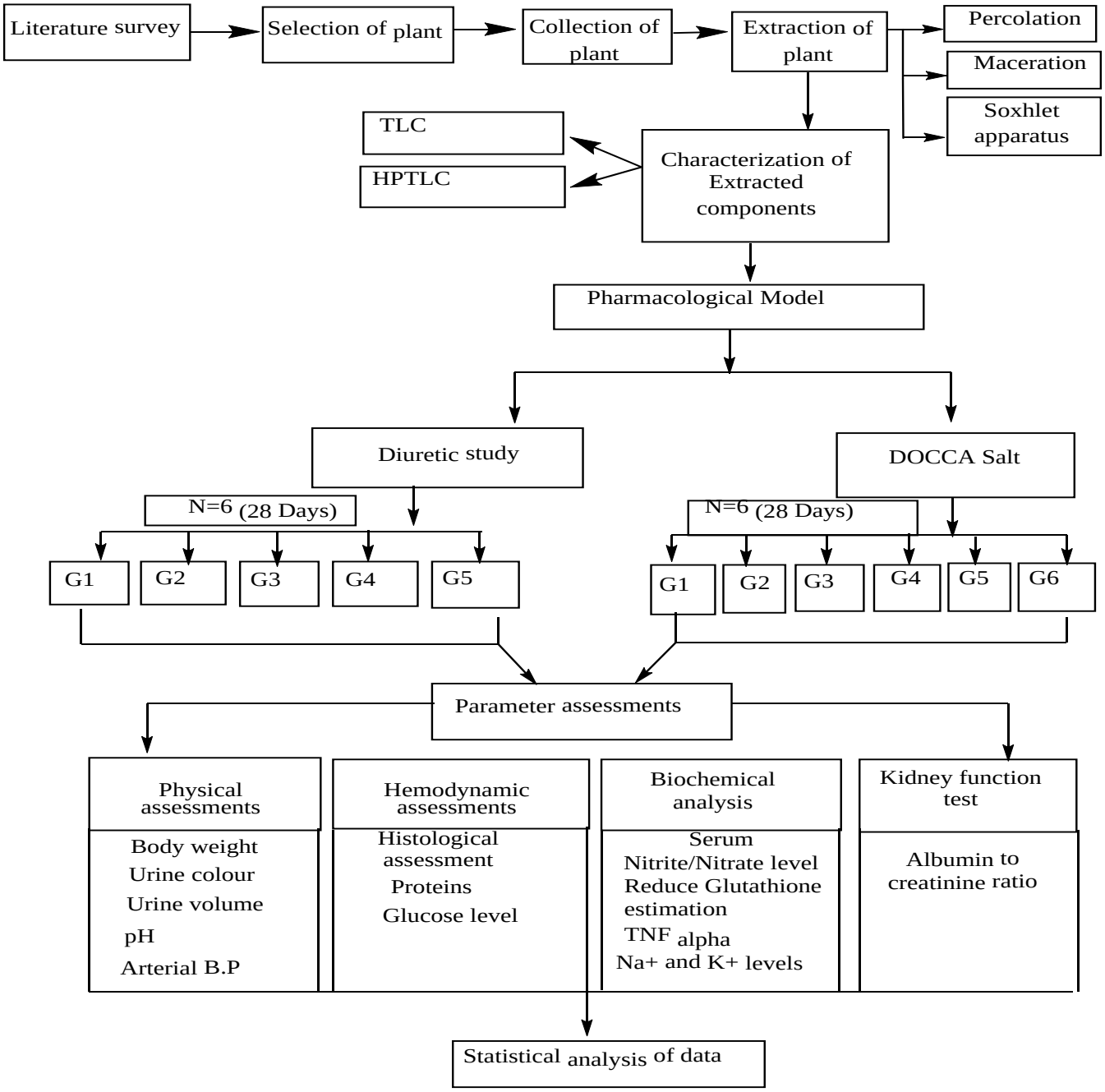
Table.8 Parameter to assessed

S.No.	Parameters
1	**Physical assessments:** • Body weight • Mean arterial B.P
2	**Hemodynamic assessments:** • Histological assessment • Glucose level
3	**Biochemical analysis:** • Serum Nitrite/Nitrate level • Reduce Glutathione estimation • TNF alpha • Na/Cl level • pH
4	**Kidney fuction test** • Albumin to creatinine ratio

9. Statistical Analysis of data.

All data will be expressed with the help of Statcistical Software Graph Pad **Prism-8.**

Tentative Chapter Plan



*TLC = Thin Layer Chromatography, HPTLC =High Performances Thin Layer Chromatography, DOCCA = desoxycorticosterone acetate, N= Number, G = Group, TNF alpha =Tumour necrosis factor alpha, Na+ = Sodium, k+ = Potassium.

Outcomes: The aim of my study is Phytochemical screening and evaluation of the diuretic activity of seed extract of *Quercus leucotrichophora* **.**

Diuretic are the drugs that physiologically used for the renal fluid regulation by excretion of water and electrolytes. Due to this, diuretics are used in the treatment of various cardiovascular diseases (congestive heart failure, hypertension), renal failure and cirrhosis. As per the literature it is clear that Quercus leucotrichophora traditional use as diuretic. But no experimental work was found on diuretic activity of Quercus leucotrichophora. So the expected outcome of present study is that the Quercus leucotrichophora the play a beneficial role in the treatment of various cardiovascular diseases (congestive heart failure, hypertension), renal failure and cirrhosis.

The other expected outcome of proposed study is that the synthetic diuretics exhibit various adverse effects and side effects, these adverse effects and side effects overcome by using natural origin diuretics and the nature origin drugs are cheaper than the synthetic drugs.

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Signature of Candidate **Signature of Supervisor**